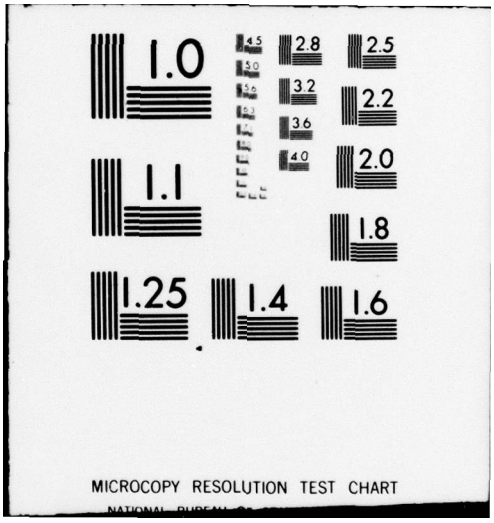
AD-A063 826 MASSACHUSETTS UNIV AMHERST DEPT OF BIOCHEMISTRY F/G 6/1
CHEMICAL ISOLATION AND IDENTIFICATION OF PHEROMONES IN THE PREP--ETC(U)
DEC 78 A M GAWIENOWSKI DAHC04-75-G-0073

UNCLASSIFIED ARO-12729.5-L NL

1 OF 1
AD
A063826

END
DATE
FILMED
3-79
DDC


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
MICROCOPY RESOLUTION TEST CHART

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

(10) (18) ARO (19) 12729.5-C

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

(6) Chemical Isolation and Identification of Pheromones in the Preputial Gland and Other Secretions of the Rat,

5. TYPE OF REPORT & PERIOD COVERED

(9) FINAL rept. 1 Dec [redacted]74 - 31 May [redacted]78,

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

(10) Gawienowski, Anthony M.

8. CONTRACT OR GRANT NUMBER(s)

(15) DAHC04-75G-0073

9. PERFORMING ORGANIZATION NAME AND ADDRESS

Department of Biochemistry
University of Massachusetts
Amherst, MA 01003

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

Research Summary

11. CONTROLLING OFFICE NAME AND ADDRESS

U. S. Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709

(11) 12 Dec [redacted]78

12. REPORT DATE

13. NUMBER OF PAGES

(12) 8p.

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report)

Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DDC RECEIVED JAN 26 1979 C

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Sex attractants, preputial gland, coagulating gland, pheromones

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Our research included the chemical analysis of the sex attractants found in the male and female rat preputial glands. The preputial gland analyses by solvent extraction, vacuum distillation, gas-chromatography and mass spectrometry led to the discovery of a number of compounds which had sex attractant properties. We found the alkyl and alkenyl acetates to be mainly sex attractants for the female rat. The male rats were primarily attracted to sulfur containing molecules. Our bioassay apparatus was modified and improved

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified 405 593

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

AD A063826

DDC FILE COPY

20. Continued

to allow its use outdoors. When this waterproof bioassay model was utilized for rat sex attractant research, we found that many of the odorants preferred by tame laboratory rats were also attractive to wild rodents under field conditions.

We reported that the voided urine of intact male rats has an avoidance effect on the normal adult male. This quality was not present in bladder urine of adult males, nor in voided urine of castrated or immature males. The avoided substance can be extracted with ethyl ether from normal voided urine. Our research indicates that the marking pheromone is produced by the androgen controlled coagulating glands and released into the urine during urination.

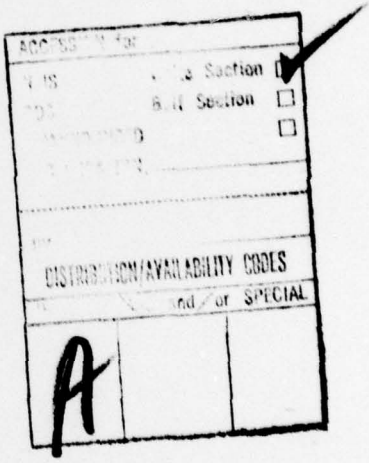


TABLE OF CONTENTS

FOREWORD

The research report mainly utilizes chronologically the published results. Therefore, a short report is given on the various papers which are included in the appendix section.

Recently, our research paper entitled "Bioassay Apparatus for Rodent Olfactory Preferences Under Laboratory and Field Conditions" by A. M. Gawienowski et al. was accepted for publication by the Journal of Chemical Ecology. It describes our modified bioassay apparatus which automatically registers frequency and the total time that rodents investigate sample odors. The apparatus provides free access for wild rodents, and its rugged weatherproof construction allows outdoor use for extended time periods. The photocell light source is covered by an infrared filter, and it can be operated continuously or for preset time intervals.

When the outdoor apparatus was utilized for rat sex attractant research, many of the odorants preferred by tamed laboratory rats were also attractive to wild rodents under field conditions. Among the compounds that appeared to be most effective under field conditions, hexanal and arachidonyl acetate were also attractive to the laboratory rats of both sexes. Decanoic acid and dimethyl sulfite were preferred by the male laboratory rats, while pentyl acetate and 2-acetyl-pyridine evoked a significant response in female laboratory rats. A good, although less dramatic attraction was found under preliminary field conditions for acetal, 4-methyl-3-pentene-2-one, decanol, dimethyl disulfide, anisole and hexyl mercaptan.

The outdoor bioassay apparatus can provide useful information on olfactory preferences both in laboratory and field experiments. Under field conditions the evaluation of area repellants or attractants for toxic baits in wildlife management could be performed by use of this apparatus with relative ease.

One phase of our research resulted in the publication "Impairment of Olfactory Perception in Male Rats by Treatment with p-Chlorophenylalanine and Hydrocortisone", by A. M. Gawienowski, P. J. Orsulak and M. Stacewicz-Sapuntzakis. This article will soon be published in Endocrine Research Communications. We found p-chlorophenylalanine (PCPA) treatment eliminated the normal male rat preference for the female preputial odor. Oral administration of hydrocortisone had a similar effect on dimethyl sulfite preference, which slowly reappeared after discontinuance of the treatment.

Our experiments were designed to observe and compare male rat responses to attractive odors after the administration of PCPA or hydrocortisone in order to explain their behavioral effects and gain a better understanding of the olfactory processes.

After the PCPA treatment the rats became lethargic and less interested in the sample odors. Their responses to the female preputial extract (sex attractant odor) and control odor did not differ significantly, although before the PCPA treatment these animals demonstrated a significant preference for the female preputial odor. The abolition of the male rat response to the female preputial odor indicates that the discrimination of olfactory cues in the PCPA treated animals apparently was impaired. This may partially explain their enhanced homosexual behavior. PCPA is believed to stimulate their mounting behavior by the depletion of brain serotonin.

Hydrocortisone treatment did not lower the general mobility of the animals, as they continued to investigate both samples. However, their preference for dimethyl sulfite disappeared during the treatment. The recovery of pre-treatment

behavior was gradual, and the return to a significant preference was noted one week after the termination of hydrocortisone administration.

There is some evidence from the study of Addison's disease patients suggesting a close connection between cortical hormone levels and olfactory acuity. The decrease in endogenous adrenal cortical hormone levels apparently results in a large increase in olfactory sensitivity which decreases to normal after adrenal cortical hormone treatment. Our experiment indicates that the administration of hydrocortisone to healthy animals has a similar effect. The decrease of olfactory acuity after hydrocortisone treatment may be related to serotonin depletion, produced by the enhanced tryptophan pyrrolase activity, which diverts tryptophan metabolism from the serotonin pathway toward kynurenin production. PCPA is known to block the synthesis of 5-hydroxytryptophan from tryptophan in the serotonin pathway.

The results of our experiments with PCPA and hydrocortisone suggest that brain serotonin level may regulate olfactory sensitivity and hence many aspects of animal behavior dependent on the sense of smell. The effect on the olfactory processes should be observed when testing new pharmaceuticals, solvents or related compounds.

This observation of the adrenal cortical hormone influence on olfaction might be utilized by the U. S. Army for the screening of dogs to be used for mine detection. The dogs with lower adrenal cortical hormone secretion levels probably would be best for odor detection purposes.

One aspect of our sulfur compound research entitled "Attraction of Rats to Sulfur Compounds" was published in Behavioral Biology 23:267-270. 1978. We assayed adult male and female rats for their olfactory preference of various sulfur-containing compounds. Both sexes preferred dimethyl disulfide. Male rats were also interested in the dimethyl sulfite and hexyl mercaptan odor while females preferres methyl isothiocyante. The three latter compounds were found to be present in rat preputial gland volatiles and may aid in sex recognition.

As mentioned in our 7/1/77--12/31/77 semi-annual report of ARO proposal number 127292, we investigated the reaction of male and female rats to thiols of increasing molecular weight. Thiols were found to attract only male rats (pentyl, hexyl, heptyl, octyl, decyl and toluyl). The females were indifferent to most of the thiol compounds; however. repulsion was noticed only in the case of the 2-octane thiol compound.

A review of our previous work and related mouse preputial work was entitled "Chemical Attractants of the Rat Preputial Gland" by A. M. Gawienowski. It appeared in'Chemical Signals in Vertebrates', edited by D. Mueller-Schwarze and M. M. Mozell, 1977, Plenum Publ. The article reviewed the evidence that the lipid fraction of the preputial gland contained attractants for the opposite sex. One possible source for the sex attractants was the alkyl acetates in the preputial gland. Spener et al. (1969), reference in the paper, reported that size, lipid content and relative amounts of alkyl acetates in female mice preputial glands were increased by testosterone injections.

Our research on the volatile compounds of the male rat preputial gland extracts by gas chromatography and mass spectroscopy revealed n-aliphatic acetates. Female rats preferred the odor of various saturated and unsaturated acetates, while the male rats were repelled by or indifferent to most of these compounds. Our bioassay apparatus recorded the number of approaches and the investigation time for each animal offered the test compounds.

It is possible that n-aliphatic acetates contribute to the sex-specific odor of rat preputial gland secretions. The differences in their relative content combined with the sex dependent receptivity of the animals suggests that the acetates may act as sex pheromones for the female rat. This work was published in J. Chem. Ecology 3:411-417, 1977 and entitled, "Rat Olfactory Response to Aliphatic Acetates", by M. Stacewicz-Sapuntzakis and A. M. Gawienowski.

In the paper, "Attractant Effect of Female Preputial Gland Extracts on the Male Rat", in Psychoneuroendocrinology 1:411-418, 1976, by A. M. Gawienowski et al. some earlier extraction details are given. Extraction of attractive compounents with different solvents revealed biological activity which was connected with relatively nonpolar, volatile lipid substances. Fatty acids were excluded as active components. The volatile lipids were partially analyzed by gas chromatography, and the active compounds were found in a specific fraction.

Our laboratory group also investigated the marking pheromone of the rat urine. We found the voided urine of intact rats had an avoidance effect on the normal male. The compound(s) was not present in bladder urine of adult males, nor in voided urine of castrated or immature males. The avoided substances were extracted with ethyl ether from normal male voided urine. This suggested that the marking pheromone was produced by an androgen controlled gland(s) and released into the urine during urination. This work was published in 'Hormones and Behavior' 7:401-405, 1976, by A. M. Gawienowski et al. and entitled, "Androgen Dependence of a Marking Pheromone in Rat Urine".

We repeated the above type of experiment and used castrated rats and castrated rats treated with testosterone. The castrated rats treated with testosterone did synthesize the marking pheromone because we found it in the voided urine. This work was submitted for publication.

While at the Oak Ridge Associated Universities on sabbatical leave, I started to analyze the lipids of the rat coagulating gland. This work is in a very preliminary stage and should be ready for manuscript form in about six months. As far as we know, no one has ever analyzed the coagulating gland lipids.

Our research paper entitled, "Presence of Sex Pheromone in Preputial Glands of Male Rats", by A. M. Gawienowski, et al. was published in J. of Endocrinology 67:283-288, 1975. We found the female rats consistently preferred the odor of the male rat preputial gland compared with that of foot pads, submaxillary-sublingual glands, coagulating glands, liver, fat or muscle. Both saline homogenates and ether extracts were effective. Female rats did not respond to the odor of female preputial extract, and they preferred the odor of normal male preputial extract to that from castrated rats.

The sex attractant was not associated with the fatty acids of the preputial extract. The fractionation of the volatile components of preputial extracts

by gas chromatography revealed that most of the biological activity resided in a specific fraction.

We have continued research on the volatile sex attractants of the male preputial glands utilizing a glass capillary column in our modified gas chromatograph. This work is only in the preliminary stages.

BIBLIOGRAPHY

Gawienowski, A. M., Berry, I. J. and Kennelly, J., Coagulating Glands as a Source of Rat Aversion Pheromone (submitted for publication) 1978.

Gawienowski, A. M., Orsulak, P. J. and Stacewicz-Sapuntzakis, M., Impairment of Olfactory Perception in Male Rats by Treatment with a p-Chlorphenylalanine and Hydrocortisone, Endocrine Research Communications (in press) 1978.

Gawienowski, A. M., Keedy, D. A. and Stacewicz-Sapuntzakis, M., Bioassay of Rodent Olfactory Preferences in Laboratory and Field Conditions, J. Chemical Ecology (in press) 1978.

Gawienowski, A. M. and Stacewicz-Sapuntzakis, M.. Attraction of Rats to Sulfur Compounds, Behavioral Biol. 23, 267-270, 1978.

Katz, M. L., Blanchette, M. and Gawienowski, A. M., Effects of the Odorant Amyl Acetate on the Estrous Cycle of the Rat, Fifth New England Endocrinology Conf., Oct. 8, 1977, U. of Mass., Amherst (abstract).

Katz, M. L., Siwek, D. F. and Gawienowski, A. M., Microscopic Quantitation of ^{3}H-2-Deoxyglucose Concentration Reveals Effects of Adrenal Cortical Hormones on Regional Brain Metabolism with Specific Attention to the Olfactory Bulbs and Suprachiasmatic Nucleus, Fifth New England Endocrinology Conf., Oct. 8, 1977, U. of Mass., Amherst (abstract).

Gawienowski, A. M., Chemical Attractants of the Rat Preputial Gland, Symposium on Chemical Signals in Vertebrates, Saratoga Springs, N. Y., June, 1976, invited paper, chapter of the book "Chemical Signals in Vertebrates". ed. D. Müller-Schwarze and M. Mozell, Plenum Press, N. Y., 1977.

Stacewicz-Sapuntzakis, M. and Gawienowski, A. M., Rat Olfactory Response to Aliphatic Acetates, J. Chem. Ecology 3, 413-419, 1977.

Gawienowski, A. M., DeNicola and Stacewicz-Sapuntzakis M., Androgen Dependence of a Marking Pheromone in Rat Urine, Hormones and Behavior 7, 401-405, 1976.

Gawienowski, A. M., Orsulak, P. J., Stacewicz-Sapuntzakis, M. and Pratt, J. J., Jr. Attractant Effect of Female Preputial Gland Extracts on the Male Rat, Psychoneuroendocrinology 1, 411-418, 1976.

Gawienowski, A. M., Orsulak, P. J., Stacewicz-Sapuntzakis, M. and Joseph, B. M., Presence of Sex Pheromone in Male Rat Preputial Gland, J. Endocrinology 67, 283-288, 1975.

Grosso, D. S. and Gawienowski, A. M., Inhibition of Mitochondrial Monoamine Oxidase of the Rat Uterus and Liver by Clorgyline, Pargyline and Harmline, Biochemical Pharamacology 25, 957-961, 1975.

Joseph, B. M. and Gawienowski, A. M., Pheromones of the Male Preputial Gland, New England Endocrinology Conference, Second Annual Meeting, Storrs, CT, Dec. 6, 1974 (abstract).